UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/553,097	10/13/2005	Paul King	NREL 03-11	4772

7590 04/04/2008

Paul J White
Nrel
1617 Cole Boulevard
Golden, CO 80401

EXAMINER	
CHOWDHURY, IQBAL HOSSAIN	
ART UNIT	PAPER NUMBER
1652	

MAIL DATE	DELIVERY MODE
04/04/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/553,097	KING ET AL.	
	Examiner	Art Unit	
	IQBAL H. CHOWDHURY	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 January 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,30-42 and 45* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 30-42, 45* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Application Status

Claims 1, 30-42 and 45 are currently pending in this application.

In response to a previous Office action, a final action (mailed on October 12, 2007), Applicants filed a response and amendment received on January 10, 2008 amending claims 1, 35, 40, and canceling claims 27-29 and 43-44, is acknowledged.

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on January 10, 2008 has been entered.

Claims 1, 30-42 and 45 are under consideration and will be examined herein.

Applicants' arguments filed on January 10, 2008, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Withdrawn-Claim Rejections - 35 U.S.C. § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Previous rejection of Claims 1 and 30-42, 45 under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of "identified amino acid residues" is withdrawn in view of

applicants amendment of claim 1 by further limiting the above phrase by adding limitation of "tryptophan, isoleucine, leucine, phenylalanine and derivatives thereof".

Previous rejection of claims 40-22 and 45 under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of "a derivative of an oxygen-sensitive iron hydrogenase", is withdrawn in view of amendment of claim 40 by deleting "derivative" from the above phrase.

Previous rejection of Claims 40-42 and 45 under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of "an oxygen-resistant iron hydrogenase from green algae or cyanobacteria --- wherein one or more residues in the in the oxygen-resistant are substituted", is withdrawn in view of amendment of claim 40 by inserting "oxygen-sensitive iron hydrogenase", which overcome the rejection.

Previous rejection of Claims 1 and 30-34 under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of "the oxygen-sensitive iron hydrogenase" (line 3), which lacks antecedent basis, is withdrawn in view of applicants amendment of claim 1.

Previous rejection of Claims 36-39 under 35 U.S.C. 112, second paragraph, as being indefinite in the recitation of proteins, which are outside the scope of claim 35 that recites a naturally occurring protein i.e. "An oxygen-resistant iron hydrogenase from green algae or cyanobacteria", is withdrawn in view of applicants amendment of claim 35.

Previous rejection of Claims 33 and 35 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn in view of applicants amendment of claims and persuasive arguments.

Previous rejection of Claims 43-45 under 35 U.S.C. 112, second paragraph, as being

indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn in view of applicants cancellation of claims 43 and 44.

Maintained - Claim Rejections - 35 U.S.C. § 112 (1st, Written description)

Previous rejection of Claims 1, 30-42 and 45 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is maintained. This rejection has been discussed at length in the previous Office action. The rejection is maintained as for the following reasons.

Claims 1, 30-42 and 45 are directed to any oxygen-resistant iron hydrogenase derived from green algae by substitution of one or more identified amino acid residues within a hydrogen channel of the oxygen-sensitive iron hydrogenase.

Applicants argue that "they have reduced to practice the ability, to construct oxygen-resistant hydrogenases using computer modeling to identify H_2-channel residues suitable the substitution by all amino acid having properties that limit 0_2-diffusion through the channel, then generating host cells transformed with such oxygen-resistant hydrogenases and produced two oxygen-resistant mutants with V240W substitutions capable of increased production of H_2. Applicants also argue that Figure 1A shows a protein alignment of 5 iron hydrogenases from *Clostridium, Desulfovibrio,* and *Chlaydomonas* and the sequence alignments demonstrate a high overall sequence identity and even greater sequence identity between the residues forming the channel and by demonstrating sequence alignments with the 5 iron hydrogenases, with desirable sequence identities, Applicants provide sufficient description relating to how iron hydrogenases

from a variety of species can be subjected to the same procedures. Applicants further argue that Figure 1B contains homology models of HydAl and HydA 1 superimposed on Cpl, which demonstrates the ability to identify, H2-channels and active sites, the catalytic core region of CpI is aligned with the protein sequence of HydA1 (Figure 2), and contains a number of similar and identical residues found in the HydAl sequence indicating the catalytic core region of HydAl. Furthermore, Figures 3 and 4 show predicted H2- channel structures in wild-type and mutant HydAl and demonstrate the narrowing of the H2-channel the substitution of specified amino acids with bulky amino acids, and Figure 5 demonstrates that the HydA1 cDNA genomic insert having the V240W mutation was present in the transformed *C. reinhardtii.* Applicants provide the sequences of several H2- channels as well as the sequence of each motif and the motifs contain a series of cysteine residues shown to have functional activity within the catalytic center. These models depict the structural change when specified amino acids are substituted with amino acids capable of narrowing the channel diameter. Applicants further argue that they have described an iron hydrogenase as an enzyme having three highly conserved motifs with conserved cysteine residues - these motifs should share at least 90% homology with a known hydrogenase such as CpI. Thus, iron hydrogenase variation within the green algae genus is actually quite small".

The Examiner acknowledges applicants lengthy arguments and amendments, however, claim 1 still reads on a genus of "oxygen resistant any iron hydrogenase derived from any green algae oxygen-sensitive any iron hydrogenase by substitution mutation of one or more residue within a hydrogen channel", which is extremely broad because the claim interprets any iron hydrogenase having no structure with oxygen-resistant properties from oxygen-sensitive any iron

hydrogenase having any structure from any green algae (which includes a large group of algae), which encompasses many mutants, variants and recombinants, wherein the mutant (oxygen-resistant) and wildtype iron hydrogenase structures are not defined that is required for fulfilling the written description requirement, i.e. structure and function relationship as such a skilled artisan could practice the claimed invention. While the specification and the figures, provides some guidance, however, said information cannot be the representative of the entire genus, and one of ordinary skill in the art cannot practice claimed invention without knowing the structural feature of the claimed genus commensurate with functional feature. As discussed in the written description guidelines the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species, which are adequately described are representative of the entire genus. **Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.** Satisfactory disclosure of a representative number depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of species disclosed. For inventions in an unpredictable art, adequate written description of a genus, which embraces widely variant species, cannot be achieved by disclosing only one species within the

genus. The specification teaches a two representative species of SEQ ID NO: 6 (HydA1) and SEQ ID NO: 7 (CpI) of said iron hydrogenase. The genus of polypeptide of iron hydrogenase whether oxygen-sensitive or resistant, is structurally diverse as it broadly encompasses many mutants, variants and recombinants comprising hydrogenase activity having different structures. As such, the disclosure solely of functional features present in all members of the genus is insufficient to be representative of the attributes and features of the entire genus. Therefore, the rejection is maintained.

Maintained - Claim Rejections - 35 U.S.C. § 112 (1st, Scope of enablement)

Previous rejection of Claims 1, 30-42 and 45 under 35 U.S.C.. 112, first paragraph, enablement requirement, is maintained. This rejection has been described at length in previous Office Action. The rejection is maintained for the following reasons.

Applicants in the lengthy remarks argue that "claims as amended now indicate the iron hydrogenase is from green algae and green algae iron hydrogenases are enabled by the specification and figure 1A, which displays the iron hydrogenase sequence alignments for three bacteria (two clostridia, one Desulfovibrio) and green algae, and Figure IB which shows a green algae hydrogenase structural model superimposed with a clostridium hydrogenase model, and Figure 2 which displays the sequence alignment of a clostridium hydrogenase core sequence with a green algae protein, which indicates the sequence identity for iron hydrogenase family proteins is at least 66%, which teaches the structural characteristics of all iron hydrogenases, which describes the conserved motifs identified in all iron hydrogenase family

members, and Examples 1-3 which apply these teachings and demonstrate the production of a green algae oxygen-resistant iron hydrogenase.

This is not found persuasive because contrary to applicants arguments, claims still read on oxygen-resistant any iron hydrogenases derived from any green algae, wherein said iron hydrogenase derived from oxygen-sensitive any iron hydrogenases by substituting any identified one or more amino acid residues within its hydrogen channel with either tryptophan, isoleucine, leucine, phenylalanine or derivatives thereof. To practice the claimed invention, one of ordinary skilled in the art should know how to make the claimed invention, i.e. structural feature of oxygen sensitive any iron hydrogenase to make desired mutation for making oxygen-resistant iron hydrogenase, which would require undue experimentation. Besides, mutating identified amino acid residues, wherein said identified amino acid residues are not defined which would also require enormous number of undue experimentation.

Applicants also argue that Applicants identify the residues lining the H2-channel by comparing a HydAl sequence to a known iron-hydrogenase, CpI (Figures 1-4) and the conserved residues are seen in these same figures, and used in conjunction with computer modeling to determine which residues if substituted would most affect channel diameter. Example 1 demonstrates the use of computer modeling to provide approximate HydA1 structure and 1-12-channel environment. When a selected amino acid is replaced with a bulky amino acid, the modeling software predicts the effects on the channel environment and diameter. Further, the Examples demonstrate Applicants' knowledge in the way the substitution of an amino acid residue lining the H2-channel affects channel diameter (structure) and hydrogen production in the presence of oxygen (function). Thus, the level of predictability is such that one of skill in the

art would be capable of making and using oxygen-resistant hydrogenases within the scope of the claims. Applicants further, argue that they teach that an unknown hydrogenase is first compared to a known hydrogenase to identify the H2-channel and conserved regions. This procedure is well known to one skilled in the art and requires sequencing the unknown hydrogenase and using readily available software programs to perform sequence alignments with the known hydrogenase and use of computer modeling to identify those residues projecting into the H2-channel as candidates for substitution with a bulky amino acid. Applicants describe *in silico* testing to determine the potential of a particular substitution to reduce channel diameter. Thus, Applicants provide all the information and more necessary for one skilled in the art to practice the subject matter of the claims without undue experimentation. Therefore, Applicants' state that claims are enabled by the specification for at least the following reasons: they are commensurate in scope with the specification, the level of predictability is such that candidate residues for substitution with bulky amino acids are readily identified and the resulting functional/structural change foreseen, and the amount of direction provided by the inventors is sufficient to enable one of ordinary skill to practice the claimed subject matter without undue experimentation.

Applicant's arguments have been fully considered but are not deemed persuasive to overcome the rejection on enablement issues. The examiner acknowledges the amendment to the claims but disagrees with the applicant's contention that the claimed invention is sufficiently enabled to the full scope of the claims. The specification, while being enabling for an oxygen resistant iron hydrogenase obtained by substitution of amino acids alanine (A) at positions 78, 244, 248; valine (V) at position 240; glycine (G) at position 86 and leucine (L) at position 93, which are lining the hydrogen channel with Trp, Ile, Leu, or Phe in the HydA1 dehydrogenase,

which is oxygen-sensitive (isolated from **Chlamydomonas reinhardtii**) for use in the production of hydrogen gas, does not reasonably provide enablement for any such oxygen-resistant mutant iron hydrogenases derived from any or all oxygen-sensitive iron hydrogenases isolated from any algae in the group of green algae or any cyanobacteria and said iron-resistant hydrogenase is derived by substituting one or more identified amino acid residues within the hydrogen channel with recited amino acid residues. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, **to make and/or use** the invention commensurate in scope with these claims.

As mentioned in the previous Office Actions, Claims 1, 30-42 and 45 are so broad as to encompass oxygen-resistant any iron hydrogenases derived from any green algae wherein said iron hydrogenase derived from oxygen-sensitive any iron hydrogenases by substituting one or more amino acid residues within its hydrogen channel with recited amino acid residues. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of oxygen-resistant iron hydrogenase and the different amino acids for substitution broadly encompassed by the claims. The genus of mutant polypeptides required to practice the claimed invention is a very large genus with the potentiality of being highly structurally variable genus. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number iron hydrogenase including many mutants and variants broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in

the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. The specification discloses only a few mutants of a single hydrogenase from only **Chlamydomonas reinhardtii,** which is insufficient to adequately describe the required genus having these specific functional characteristics.

Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including any oxygen-resistant iron hydrogenases derived from any oxygen-sensitive iron hydrogenases by substituting one or more amino acid residues within the hydrogen channel of any oxygen-sensitive iron hydrogenases from any source. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of oxygen-resistant any iron hydrogenases isolated from any algae, wherein said oxygen-resistant hydrogenase derived from oxygen-sensitive hydrogenase by substituting one or more identified amino acid residues within the hydrogen channel with recited amino acids such that said variant has the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). Therefore, the rejection is maintained.

Maintained-Claim Rejections - 35 USC § 102

Previous rejection of Claims 1, 27-29, 31-42, and 45 under 35 U.S.C. 102(b) as being anticipated by Dillon et al. (US PGPUB 2007/0009942 A1, publication 1/11/2007, claim priority

of US copending application 10/411,910 filed on 4/12/2003) is maintained. It is maintained for the following reasons. Instant claims are directed to any oxygen-resistant iron hydrogenase isolated from Chlamydomonas reinhardtii any oxygen sensitive iron hydrogenase by the substitution of one or more amino acid residues including tryptophan, isoleucine, leucine or phenylalanine within the hydrogen or gas channel of any oxygen-sensitive iron hydrogenase.

Applicants argue that claim 1 is directed to an oxygen resistant iron hydrogenase derived from green algae or cyanobacteria by substituting one or more identified amino acid residues within a hydrogen channel. The one or more identified amino acid residues are independently substituted with an amino acid having properties that limit O_2 diffusion through the channel by reducing the diameter of the channel while allowing H_2 diffusion out of the channel. Applicants also argue that they have determined the HydA1 H_2-channel sequence and structure, strategic amino acid residues that when replaced with bulky amino acids causes the effective channel diameter to decrease such that the channel prevents passage of O_2 through the channel and in *silico* demonstrated that individual mutations and combined mutations reduced the average overall channel diameter. Applicants further argue that Dillon does not teach or suggest the targeted substitution of an identified amino acid with an amino acid that limits 0_2 diffusion through the channel while allowing H_2 diffusion out of the channel.

This is not found persuasive because first of all, Dillon et al. indeed teach a method of producing hydrogen gas in presence of oxygen by using a modified hydrogenase gene from Chlamydomonas reinhardtii, which are green algae similar to instant application. Dillon et al. also teach modification of the bulky amino acid residue in gas channel (hydrogen) motif including phenylalanine or leucine and successfully produced hydrogen in presence of oxygen

that indicates that oxygen diffusion through the channel is restricted, which irreversibly inhibits hydrogenase enzyme. Dillon et al. further teach that some of the amino acids (X) would be replaced with any amino acid, which reads on applicants desired amino acids to be replaced with the desired amino acid. Since applicants do not specify specific amino acids (identified amino acids) to be replaced, the Examiner interprets it within the scope of Dillon et al. modification.

Regarding reduction of diameter of the hydrogen or gas channel, since, the iron hydrogenase of the instant application and that of the reference is one and the same, and the same amino acids are involved in substituting of amino acid in the hydrogen/gas channel, Examiner takes the position that the hydrogen channel diameter of hydrogen channel as disclosed by the instant application would be inherently possessed by the hydrogenase protein of the reference. Since the Office does not have the facilities for examining and comparing applicants' protein diameter with the diameter of protein disclosed by the prior art, the burden is on the applicant to show a novel or unobvious difference between the claimed product diameter (i.e. protein diameter) and the product diameter of the prior art (i.e., protein diameter). See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *In re Fitzgerald* et al., 205 USPQ 594. As discussed previously, Dillon et al. teach an oxygen-resistant or tolerant iron hydrogenase derived from oxygen sensitive hydrogenase i.e. hydrogenase activity is inhibited by the presence of oxygen, capable of producing hydrogen, which also contained nickel ion in addition to iron in the active site i.e. the hydrogenase comprises iron and nickel ion having bimetallic active site. Dillon et al. also teach substitution of one or more amino acid residues in the hydrogen channel region near active site comprising $FX^1X^2X^3G^1G^2VMEA^1A^2X^4R$ region of the hydrogenase protein, wherein X can be any amino acid substituted with any amino acid. Dillon et al. further

teach substitution of amino acid phenylalanine, glycine, valine, methionine, glutamic acid, alanine, arginine and glutamine into the gas channel segment, in the amino acid sequence (abstract, p1, Col 1-2, p4, Col 1-2, p18). Therefore, the rejection is maintained.

Conclusion

Claims 1, 30-42 and 45 are rejected.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Iqbal Chowdhury, Ph.D. whose telephone number is 571-272-8137. The examiner can normally be reached on 9:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Nashaat N. Nashed, can be reached on 571-272-0934. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Respectfully,

Iqbal Chowdhury, PhD, Patent Examiner
Art Unit 1652 (Recombinant Enzymes)
US Patent and Trademark Office
Rm. 2B69, Mail Box. 2C70
Ph. (571)-272-8137, Fax. (571)-273-8137

/Iqbal H. Chowdhury, Ph.D./
Patent Examiner, Art Unit 1652